

FIG. 1A

H36.D2.B7 Anti-Tissue Factor Light Chain Variable Region

GACATTCAGATGACCCAGTCTCCTGCCTCCCAGTCTGCATCTCTGGGAGAAAGTGTACCATCACATGC
D I Q M T Q S P A S Q S A S L G E S V T I T C
CTGGCAAGTCAGACCATTGATACATGGTTAGCATGGTATCAGCAGAAACCAGGGAAATCTCCTCAGCTC
L A S Q T I D T W L A W Y Q Q K P G K S P Q L
CTGATTATGCTGCCACCAACTTGGCAGATGGGTCCCATCAAGGTTCAGTGGCAGTGGATCTGGCACA
L I Y A A T N L A D G V P S R F S G S G S G T
AAATTTCTTCAAGATCAGCAGCCTACAGGCTGAAGATTGTAAATTATTACTGTCAACAAGTTAC
K F S F K I S S L Q A E D F V N Y Y C Q Q V Y
AGTTCTCCATTCACGGTCGGTGCTGGGACCAAGCTGGAGCTGAAA
S S P F T F G A G T K L E L K

FIG. 1B

H36.D2.B7 Anti-Tissue Factor Heavy Chain Variable Region

GAGATCCAGCTGCAGCAGTCTGGACCTGAGCTGGTGAAGCCTGGGCTTCAGTGCAGGTATCCTGCAAG
E I Q L Q Q S G P E L V K P G A S V Q V S C K
ACTTCTGGTTACTCATTCACTGACTAACGTGTACTGGGTGAGGCAGAGCCATGGAAAGAGCCTTGAG
T S G Y S F T D Y N V Y W V R Q S H G K S L E
TGGATTGGATATATTGATCCTTACAAATGGTATTACTATCTACGACCAGAACTTCAAGGGCAAGGCCACA
W I G Y I D P Y N G I T I Y D Q N F K G K A T
TTGACTGTTGACAAGTCTCCACCACAGCCTTCATGCATCTCAACAGCCTGACATCTGACGACTCTGCA
L T V D K S S T T A F M H L N S L T S D D S A
GTTTATTTCTGTGCAAGAGATGTACTACGGCCCTTGACTTCTGGGGCCAAGGCACCACCTCACAGTC
V Y F C A R D V T T A L D F W G Q G T T L T V
TCCTCA
S S

* CDR regions underlined.

Antibody	Apparent K_d , M $^{-1}$	Apparent K_d , M
By ELISA		
D2	5.2×10^9	1.9×10^{-10}
I47	6.5×10^9	1.5×10^{-10}
K73	9.8×10^9	1.0×10^{-10}
K80	2.3×10^9	4.3×10^{-10}
L102	2.5×10^9	4.0×10^{-10}
L133	1.7×10^9	5.9×10^{-10}
By BLACore		
H36	<u>3.1×10^{10}</u>	<u>3.2×10^{-11}</u>
I43	2.3×10^9	<u>4.3×10^{-10}</u>
I47	3.2×10^9	<u>3.1×10^{-10}</u>
L133	4.6×10^9	<u>2.2×10^{-10}</u>
M107	1.1×10^9	<u>9.1×10^{-10}</u>

FIG. 2

Antibody Name	% Inhibition	
	Antibody Preincubated with TF/VIIa	
D1		0
D1B		1
H31		4
<u>H36</u>		<u>95</u>
I43		1
J131		7
K80		0
K82		0
K87		1
L97B		7
L101		0
L102		0
L105		0
L133		0
M5		1
M107		34

FIG. 3

Antibody Name	% Inhibition	
	TF Preincubated with Antibody Prior to Addition of VIIa	TF Preincubated with VIIa Prior to Addition of Antibody
D1	15	nd
D1B	48	12.7
H31	64	21
H36	0	0
I43	68	55
J131	38	11
K80	12	nd
K82	0	nd
K87	0	nd
L96	0	nd
L101	38	11
L102	14	nd
L105	4	nd
L133	13	nd
M5	0	nd
M107	0	nd

FIG. 4

[rhTF], nM	[H36.D2], nM	H36.D2/rhTF Molar Ratio	Clotting Time (seconds)	% Inhibition of rhTF Function
0.0048	0	0	102.3	0
	1.61	335.4	114.3	31.3
	3.23	670.8	121.3	45.8
0.023	0	0	77.6	0
	1.61	70.0	85.3	52.2
	3.23	140.0	91.1	65.2
	6.45	280.4	99.6	73.9
0.092	0	0	49.3	0
	3.23	35.1	65.8	65.2
	6.45	70.1	88.5	90.2
	12.90	140.2	113.3	95.7
0.46	0	0	32.6	0
	6.45	14.0	52.7	82.4
	12.90	28.0	80.2	96.7
	32.30	70.2	117.9	99.3
2.30	0	0	23.9	0
	16.10	7.0	47.1	94.4
	32.30	14.0	95.2	99.7
	64.50	28.0	115.3	99.9
11.52	0	0	22.2	0
	16.10	1.4	30.2	93.4
	32.30	2.8	46.0	98.8
	64.50	5.6	87.6	99.9
	161.30	14.0	114.0	100.0

FIG. 5

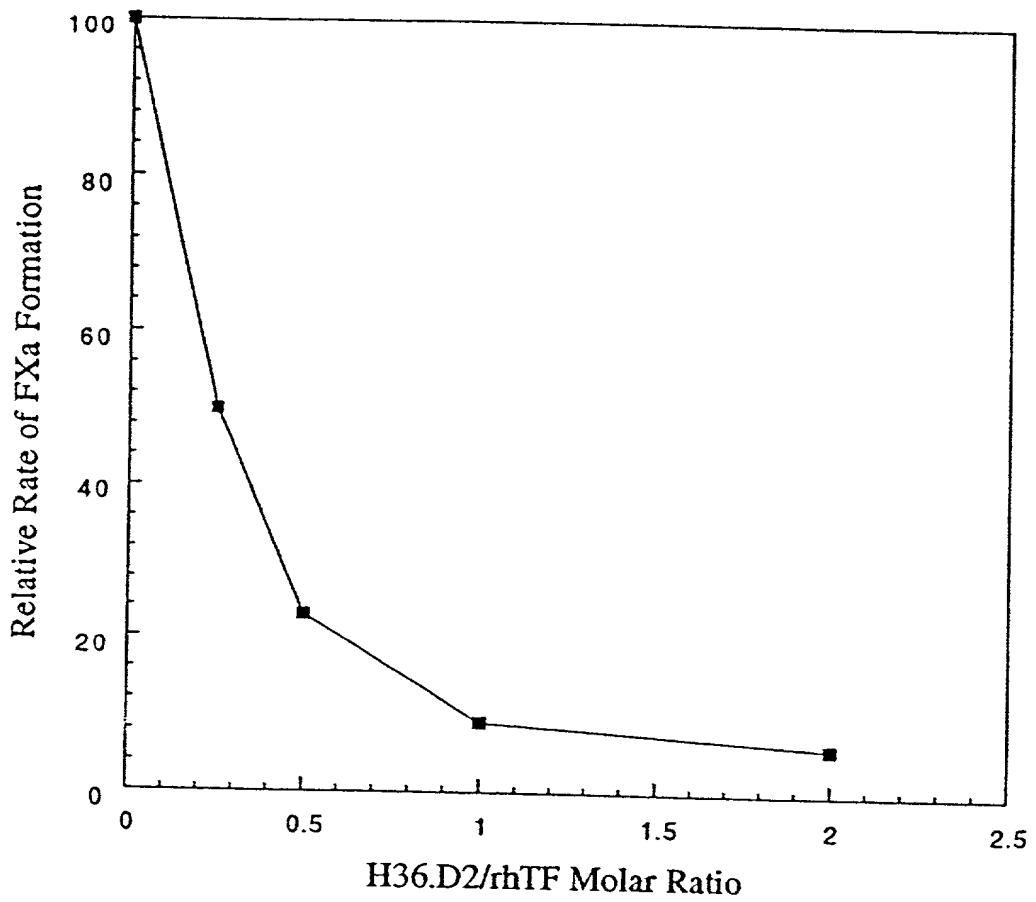


FIG. 6A

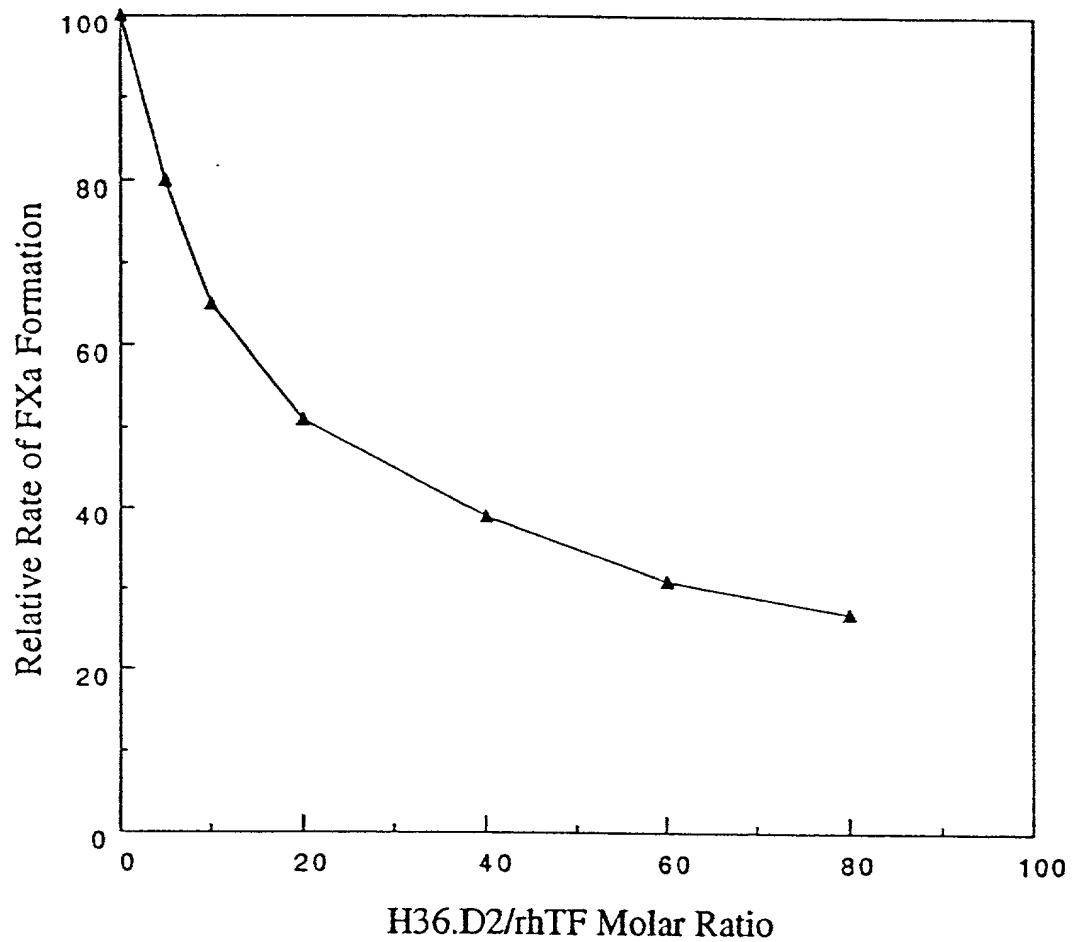


FIG. 6B

H36.D2 Concentration (ng)	<u>% Inhibition</u> Cells (TF/FVII) and H36.D2 preincubated prior to FX addition	<u>% Inhibition</u> FX and H36.D2 are added simultaneously to Cells (TF/FVII)
0	0	0
50	88	nd
100	92	nd
200	97	nd
800	nd	76
1600	nd	78
3200	nd	92

FIG. 7

2 μ l, 60ng

1 μ l, 30ng

0.5 μ l, 15ng

Native ~
8M urea
5mM DTT w

FIG. 8A

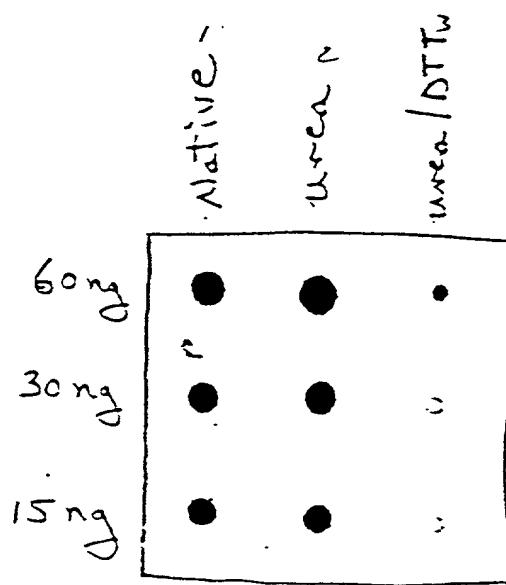
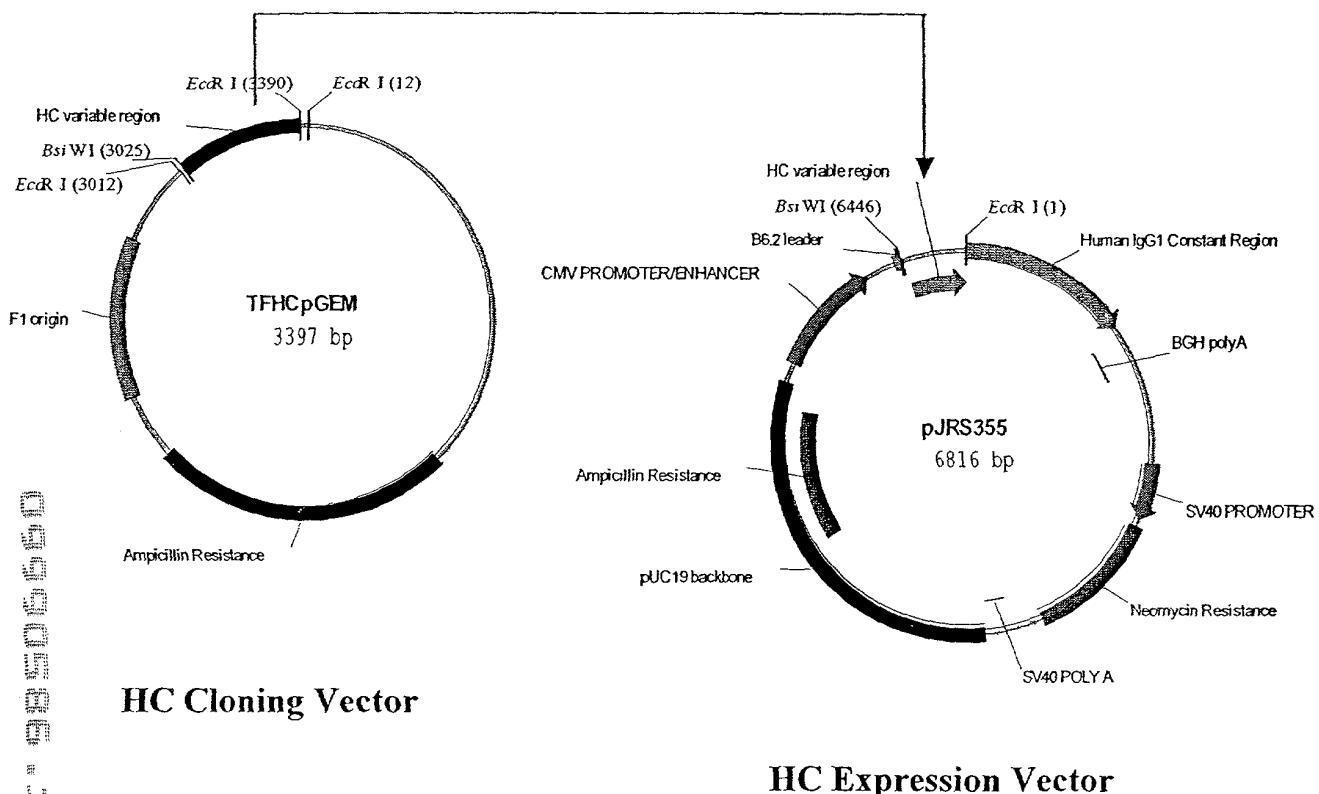


FIG. 8B

Figure A. Human IgG1-cH36 HC Variable Region Cloning and Expression Vector



HC Cloning Vector

Fig. 9A

HC Expression Vector

Fig. 9B

Figure B. Human IgG4-cH36 HC Variable Region Cloning and Expression Vector

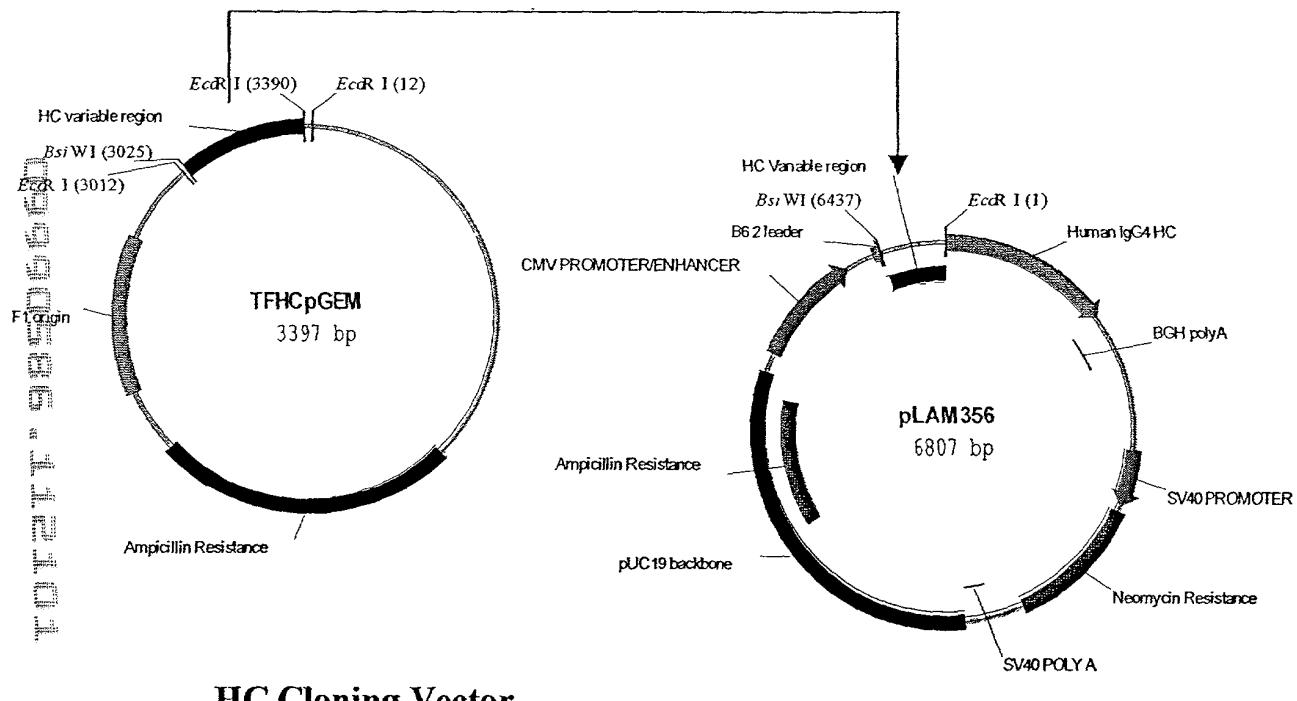


Fig. 9C

HC Expression Vector

Fig. 9D

Figure C. cH36 LC Variable Region Cloning and Expression Vector

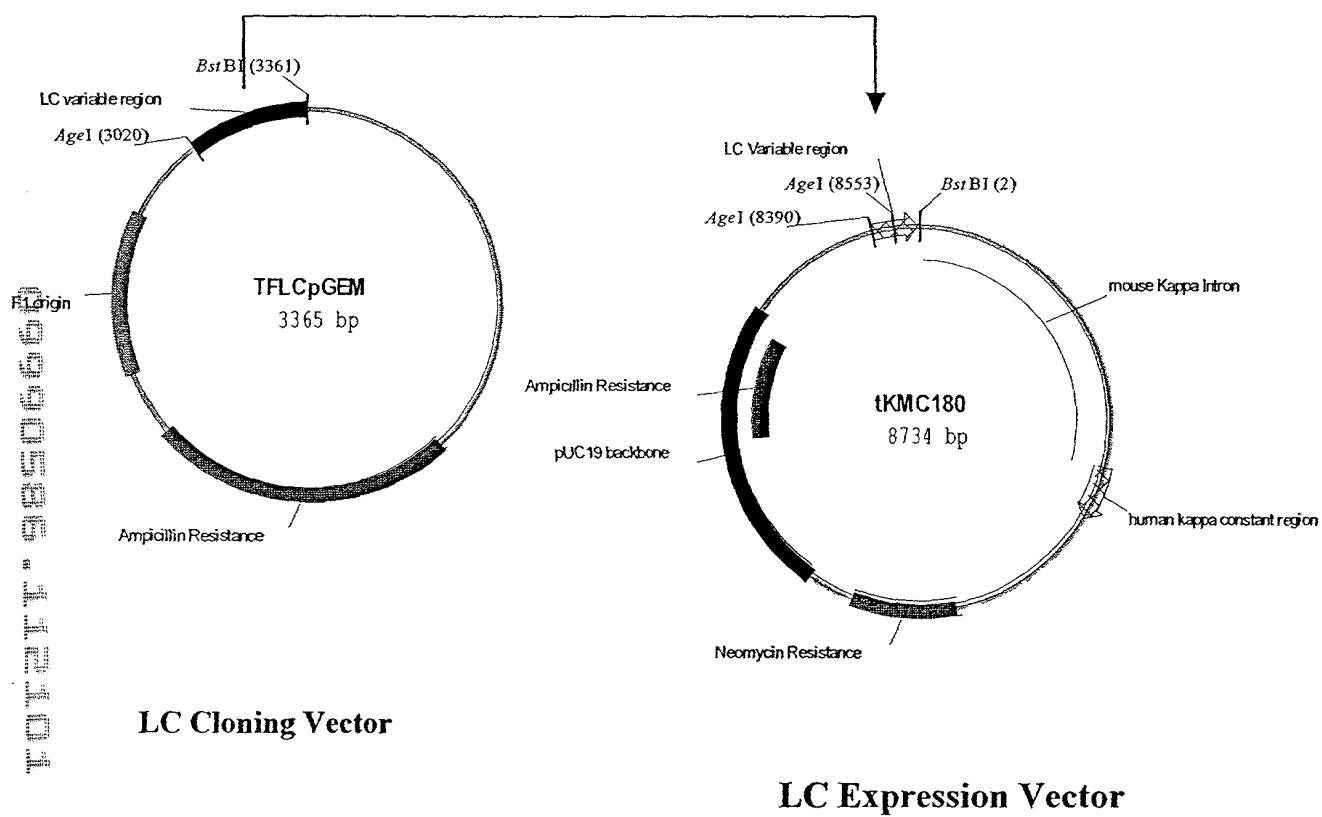


Fig. 10A

Fig. 10B

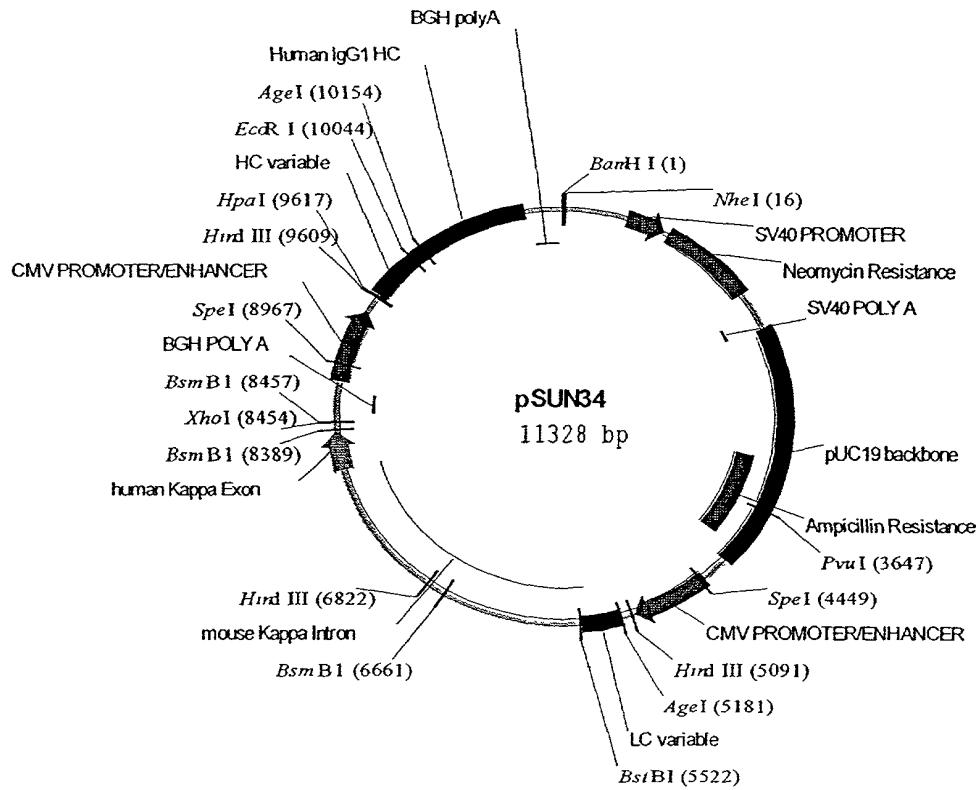


Figure D. Plasmid Map of Humanized Anti-TF IgG1 Antibody Expression Vector

Fig. 11

Humanization of anti-Tissue Factor Antibody CH36

Sequences of Partially and Fully Humanized Light Chain (LC) Variable Regions

Light Chain (LC) FR Sequences

	FR1 (23 AA)	FR2 (14 AA)	FR3 (32 AA)	FR4 (10 AA)	Names
1	10	20	35	47 57 60 70	98 107
DIQMTQSPASQSASSLGESEV T ITC	WYQQKPGKSPQLIY	GVPSRFSGSGSGTKEFSSFKISSLQ AEDFVNYYC	EGAGTKIELK	CH36-LC	
DIQMTQSPASQSASSLGESEV T ITC	WYQQKPGKSPQLIY	GVPSRFSGSGSGTKEFSSFKISSLQ	EGAGTKIELK	LC-03	
DIQMTQSPASQSASSLGESEV T ITC	WYQKPGKSPQLIY	GVPSRFSGSGSGTKEFSSFKISSLQ	EGAGTKIELK	LC-04	
DIQMTQSPASQSASS G ITC	WYQKPGKSPQLIY	GVPSRFSGSGSGTKEFSSFKISSLQ	EGAGTKIELK	LC-05	
DIQMTQSPASQSASS G ITC	WYQKPGKSPQLIY	GVPSRFSGSGSGTKEFSSFKISSLQ	EGAGTKIELK	LC-06	
DIQMTQSPASQSASS G ITC	WYQKPGKSPQLIY	GVPSRFSGSGSGTKEFSSFKISSLQ	EGAGTKIELK	LC-07	
DIQMTQSPASQSASS G ITC	WYQKPGKSPQLIY	GVPSRFSGSGSGTKEFSSFKISSLQ	EGAGTKIELK	LC-08	
DIQMTQSPASQSASS G ITC	WYQKPGKSPQLIY	GVPSRFSGSGSGTKEFSSFKISSLQ	EGAGTKIELK	LC-09	
DIQMTQSPASQSASS G ITC	WYQKPGKSPQLIY	GVPSRFSGSGSGTKEFSSFKISSLQ	EGAGTKIELK	LC-10	
DIQMTQSPASQSASS G ITC	WYQKPGKSPQLIY	GVPSRFSGSGSGTKEFSSFKISSLQ	EGAGTKIELK	LC-11	
DIQMTQSPASQSASS G ITC	WYQKPGKSPQLIY	GVPSRFSGSGSGTKEFSSFKISSLQ	EGAGTKIELK	LC-12	

Light Chain CDR Sequences of CH36

	CDR1 (11 AA)	CDR2 (7 AA)	CDR3 (9 AA)
24	L A S Q T I D T W L A	A A T N L A D	Q Q V Y S S P F T

Fig. 12B

Fig. 12C

Fig. 12D

Fig. 12A

Sequences of Partially and Fully Humanized Heavy Chain (LC) Variable Regions

Heavy Chain (HC) FR Sequences

FR1 (30 AA)	FR2 (14 AA)	FR3 (32 AA)	FR4 (11 AA)	Names
10 20	29 36 44	67 75	95 107	117 CH36-HC
EIQLQQSGPELVKPGASVQVSVCKTSGYSET	WVRQSHGKSLEWIG	KATILTVDKSSTTAFMHLNSSLTSDDSAVYFCAR	WGQGTTTVSS	
QIQLQQSGPELVKPGASVQVSVCKTSGYSET	WVRQSHGKSLEWIG	KATILTVDKSSTTAFMHLNSSLTSDDSAVYFCAR	WGQGTTTVSS	HC-01
QIQLQQSGPELVKPGASVQVSVCKTSGYSET	WVRQSHGKSLEWIG	KATILTVDKSSTTAFMHLNSSLTSDDSAVYFCAR	WGQGTTTVSS	HC-02
QIQLQQSGPELVKPGASVQVSVCKTSGYSET	WVRQSHGKSLEWIG	KATILTVDKSSTTAFMHLNSSLTSDDSAVYFCAR	WGQGTTTVSS	HC-03
QIQLQQSGPELVKPGASVQVSVCKTSGYSET	WVRQSHGKSLEWIG	KATILTVDKSSTTAFMHLNSSLTSDDSAVYFCAR	WGQGTTTVSS	HC-04
QIQLQQSGPELVKPGASVQVSVCKTSGYSET	WVRQSHGKSLEWIG	KATILTVDKSSTTAFMHLNSSLTSDDSAVYFCAR	WGQGTTTVSS	HC-05
QIQLQQSGPELVKPGASVQVSVCKTSGYSET	WVRQSHGKSLEWIG	KATILTVDKSSTTAFMHLNSSLTSDDSAVYFCAR	WGQGTTTVSS	HC-06
QIQLQQSGPELVKPGASVQVSVCKTSGYSET	WVRQSHGKSLEWIG	KATILTVDKSSTTAFMHLNSSLTSDDSAVYFCAR	WGQGTTTVSS	HC-07
QIQLQQSGPELVKPGASVQVSVCKTSGYSET	WVRQSHGKSLEWIG	KATILTVDKSSTTAFMHLNSSLTSDDSAVYFCAR	WGQGTTTVSS	HC-08
QIQLQQSGPELVKPGASVQVSVCKTSGYSET	WVRQSHGKSLEWIG	KATILTVDKSSTTAFMHLNSSLTSDDSAVYFCAR	WGQGTTTVSS	HC-09
QIQLQQSGPELVKPGASVQVSVCKTSGYSET	WVRQSHGKSLEWIG	KATILTVDKSSTTAFMHLNSSLTSDDSAVYFCAR	WGQGTTTVSS	HC-10

Heavy Chain CDR Sequences

CDR1 (5 AA)	CDR2 (17 AA)	CDR3 (8AA)	Names
31 35	50	99 66	106 CH36
D Y N V Y	Y I D P Y N G I T I Y D Q N F K G	D V T T A L D F	
31 35	50	99 66	106 HC-08
D Y N V Y	Y I D P Y N G I T I Y D Q N K G	D V T T A L D F	

Fig. 13B

Fig. 13C

Fig. 13D

FR1 (30 AA)	FR2 (14 AA)	FR3 (32 AA)	FR4 (11 AA)	Names
10 20	29 36 44	67 75	95 107	117 CH36-HC
EIQLQQSGPELVKPGASVQVSVCKTSGYSET	WVRQSHGKSLEWIG	KATILTVDKSSTTAFMHLNSSLTSDDSAVYFCAR	WGQGTTTVSS	
QIQLQQSGPELVKPGASVQVSVCKTSGYSET	WVRQSHGKSLEWIG	KATILTVDKSSTTAFMHLNSSLTSDDSAVYFCAR	WGQGTTTVSS	HC-01
QIQLQQSGPELVKPGASVQVSVCKTSGYSET	WVRQSHGKSLEWIG	KATILTVDKSSTTAFMHLNSSLTSDDSAVYFCAR	WGQGTTTVSS	HC-02
QIQLQQSGPELVKPGASVQVSVCKTSGYSET	WVRQSHGKSLEWIG	KATILTVDKSSTTAFMHLNSSLTSDDSAVYFCAR	WGQGTTTVSS	HC-03
QIQLQQSGPELVKPGASVQVSVCKTSGYSET	WVRQSHGKSLEWIG	KATILTVDKSSTTAFMHLNSSLTSDDSAVYFCAR	WGQGTTTVSS	HC-04
QIQLQQSGPELVKPGASVQVSVCKTSGYSET	WVRQSHGKSLEWIG	KATILTVDKSSTTAFMHLNSSLTSDDSAVYFCAR	WGQGTTTVSS	HC-05
QIQLQQSGPELVKPGASVQVSVCKTSGYSET	WVRQSHGKSLEWIG	KATILTVDKSSTTAFMHLNSSLTSDDSAVYFCAR	WGQGTTTVSS	HC-06
QIQLQQSGPELVKPGASVQVSVCKTSGYSET	WVRQSHGKSLEWIG	KATILTVDKSSTTAFMHLNSSLTSDDSAVYFCAR	WGQGTTTVSS	HC-07
QIQLQQSGPELVKPGASVQVSVCKTSGYSET	WVRQSHGKSLEWIG	KATILTVDKSSTTAFMHLNSSLTSDDSAVYFCAR	WGQGTTTVSS	HC-08
QIQLQQSGPELVKPGASVQVSVCKTSGYSET	WVRQSHGKSLEWIG	KATILTVDKSSTTAFMHLNSSLTSDDSAVYFCAR	WGQGTTTVSS	HC-09
QIQLQQSGPELVKPGASVQVSVCKTSGYSET	WVRQSHGKSLEWIG	KATILTVDKSSTTAFMHLNSSLTSDDSAVYFCAR	WGQGTTTVSS	HC-10

FR1 (30 AA)	FR2 (14 AA)	FR3 (32 AA)	FR4 (11 AA)	Names
10 20	29 36 44	67 75	95 107	117 CH36-HC
EIQLQQSGPELVKPGASVQVSVCKTSGYSET	WVRQSHGKSLEWIG	KATILTVDKSSTTAFMHLNSSLTSDDSAVYFCAR	WGQGTTTVSS	
QIQLQQSGPELVKPGASVQVSVCKTSGYSET	WVRQSHGKSLEWIG	KATILTVDKSSTTAFMHLNSSLTSDDSAVYFCAR	WGQGTTTVSS	HC-01
QIQLQQSGPELVKPGASVQVSVCKTSGYSET	WVRQSHGKSLEWIG	KATILTVDKSSTTAFMHLNSSLTSDDSAVYFCAR	WGQGTTTVSS	HC-02
QIQLQQSGPELVKPGASVQVSVCKTSGYSET	WVRQSHGKSLEWIG	KATILTVDKSSTTAFMHLNSSLTSDDSAVYFCAR	WGQGTTTVSS	HC-03
QIQLQQSGPELVKPGASVQVSVCKTSGYSET	WVRQSHGKSLEWIG	KATILTVDKSSTTAFMHLNSSLTSDDSAVYFCAR	WGQGTTTVSS	HC-04
QIQLQQSGPELVKPGASVQVSVCKTSGYSET	WVRQSHGKSLEWIG	KATILTVDKSSTTAFMHLNSSLTSDDSAVYFCAR	WGQGTTTVSS	HC-05
QIQLQQSGPELVKPGASVQVSVCKTSGYSET	WVRQSHGKSLEWIG	KATILTVDKSSTTAFMHLNSSLTSDDSAVYFCAR	WGQGTTTVSS	HC-06
QIQLQQSGPELVKPGASVQVSVCKTSGYSET	WVRQSHGKSLEWIG	KATILTVDKSSTTAFMHLNSSLTSDDSAVYFCAR	WGQGTTTVSS	HC-07
QIQLQQSGPELVKPGASVQVSVCKTSGYSET	WVRQSHGKSLEWIG	KATILTVDKSSTTAFMHLNSSLTSDDSAVYFCAR	WGQGTTTVSS	HC-08
QIQLQQSGPELVKPGASVQVSVCKTSGYSET	WVRQSHGKSLEWIG	KATILTVDKSSTTAFMHLNSSLTSDDSAVYFCAR	WGQGTTTVSS	HC-09
QIQLQQSGPELVKPGASVQVSVCKTSGYSET	WVRQSHGKSLEWIG	KATILTVDKSSTTAFMHLNSSLTSDDSAVYFCAR	WGQGTTTVSS	HC-10

hOAT (IgG1) Constant regions sequences

Sequences of LC constant:

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWVKVDNALQSGNSQESVTEQDSIKDSTYSSLSSSTLTLSKADYEKH
KVYACEVTHQGLSSSPVTKSFNRGEC

Sequences of HC constant:

EFASTKGPSVFLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYIC
NNNHKPSNTKVDDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMSRTPEVTCVVVDVSHEDPEVKENWYVDGVEV
HNAKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKALPAPIKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL
VKGFYPSDIAVIEWSNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Fig. 14A

Fig. 14B

hFAT (IgG4) constant region sequences

Sequences of LC Constant:

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVVDNALQSGNSQESVTEQDSDKDSTYSLSSSTLTLSKADYEK
HKVYACEVTHQGLSSPVTKSFRNGEC

Sequences of HC constant:

EFASTKGPSVFPLAPCSRSTSESTAALGCLVKDYYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSISSLVTVPSSSLGKTY
TCNVDHKPSNTKVDKRVESKYGPPCPSCPAPAEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSSQEDPEVQFNWYVDGV
EVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSL
TCLVKGFYPSDIAVEWESNGQOPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSSLSSLGK

Fig. 15A

Fig. 15B